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(71) Applicants: THE RESEARCH FOUNDATION OF STATE UNIVERSITY OF NEW YORK [US/US]; P.O. Box 9, Albany, NY 12201–0009 (US). WASHINGTON UNIVERSITY [US/US]; 660 South Euclid Avenue, St. Louis, MO 63110 (US).

(72) Inventors: KOCH, Stephen, A.; 3 Hillsview Road, Stony Brook, NY 11790 (US). WELCH, Michael, J.; 1 Spoede Lane, St. Louis, MO 63141 (US). CUTLER, Cathy, S.; 1434 Tamm Avenue, St. Louis, MO 63118 (US). QUARLESS, Duncan, A., Jr.; 319 Chester Street, Uniondale, NY 11553 (US).

(74) Agent: BARON, Ronald, J.; Hoffmann & Baron, LLP, 6900 Jericho Turnpike, Syosset, NY 11791 (US).

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(54) Title: S_3N RADIONUCLIDE COMPLEXES

(57) Abstract

A radiolabeled tris(2–mercaptobenzyl)amine complex is provided. Complexes containing analogs of the tris(2–mercaptobenzyl)amine ligand are also described. The complex is useful for diagnostic or therapeutic applications which involve localization and detection of the radiolabel in internal organs. A gallium complex can be localized in the brain.

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S_3N RADIONUCLIDE COMPLEXES

The invention was made with U.S. Government support under National Institutes of Health grant numbers CA 42925 and GM 31849 and Department of Energy grant number DE-FG 02-87-ER60512. The Government has certain rights in the invention.

The invention relates to a stable S_3N complex containing radionuclide for localization in internal organs. When the radionuclide is gallium, the complex crosses the blood brain barrier.

BACKGROUND OF THE INVENTION

Several requirements must be met by radiopharmaceuticals for uptake by and imaging of internal organs, particularly for uptake by the heart and brain. One requirement is lipophilicity. The usefulness of lipophilic ^{11}C-labeled ($t_{1/2}$=20 min) ethers and alcohols for measurement of myocardial and cerebral blood flow by PET was shown by D.D. Dischino et al., *Journal of Nuclear Medicine* **1983**, *24*, 1030-1038, and S.N. Hack et al., *Journal of Clinical Investigation* **1980**, *66*, 918-27.

A major problem encountered with gallium radiopharmaceuticals is the transchelation of Ga(III) to various iron containing proteins, particularly transferrin, which bind Ga(III) with high affinity. W.R.Harris, et al, *Biochemistry* **1983** *22*, 292-299. The radiopharmaceuticals ^{67}Ga- and ^{68}Ga-citrate actually take advantage of this transchelation by labeling transferrin *in vivo* by ligand exchange; ^{68}Ga-transferrin measures regional plasma volume. Perfusion imaging necessitates high stability gallium radiopharmaceuticals that will not undergo transchelation with transferrin.

Several Ga agents have been developed that show myocardial uptake. Green and coworkers developed a series of uncharged lipophilic Ga(III) complexes of 1,1,1-tris-(5-methoxysalicylaldiminomethyl)ethane[$(sal)_3$tame] and 1,1,1,-tris-(alkoxysalicylaldiminomethyl)ethane[$ROsal)_3$tame] as ^{68}Ga myocardial imaging agents.

M.A. Green et al., *Journal of Nuclear Medicine* **1985**, *26*, 170-180; M.A. Green, *Journal of Labelled Compounds and Radiopharmaceuticals* **1986**, *23*, 1227-1229. In an attempt to increase heart uptake, the lipophilicity of these ligands was further increased by the addition of alkoxy-substituents on the ethane backbone of the triamine framework of the tris(salicylaldimine) ligands. Increased heart uptake and higher heart:blood ratios were observed. However, the increased lipophilicity resulted in increased liver accumulation. M.A. Green et al., *The Journal of Nuclear Medicine* ***1993***, *34*, 228-233.

Ga(III) complexes of bis-aminethanethiol-cyclohexyl (BAT-TECH) ligands having 2 nitrogens and 2 sulfurs (N_2S_2) have been investigated as myocardial imaging agents. H.F. Kung et al., *Journal of Nuclear Medicine,* **1990**, *31*, 1635-1640; L.C. Francesconi et al., *Journal of the Chemistry Society, Chemical Communications,* **1991**, *Issue 2*, 94-95; See also, U.S. Patent No. 5,079,346 to Kung. The Ga(III) compounds are rapidly taken up by the heart; however, they quickly wash out and the blood level remains high, resulting in low heart:blood ratios at later timepoints.

In an attempt to design myocardial imaging agents with increased myocardial retention, Tsang and coworkers prepared a series of hexadentate bis(salicylaldimine) ligands that formed lipophilic cationic complexes with Ga(III). B.W. Tsang et al., *Journal of Medicinal Chemistry* **1994**, *37*, 4400-4406; B.W. Tsang et al., *Journal of Nuclear Medicine* **1993**, *34*, 1127-1131. These compounds, particularly Ga(III)[4,6-MeO_2sal)$_2$BAPEN]+, exhibited significant myocardial uptake with longer retention than any of the compounds mentioned previously.

It is an object of the invention to provide compounds with even longer myocardial retention times.

Attempts to develop a gallium radiolabeled agent that crosses the blood brain barrier have met with limited success. For brain imaging, the compound must be relatively small, neutral and lipophilic in order to cross the intact blood brain barrier.

A series of tris(1-aryl-3-hydroxy-4-pyridinonato)Ga(III) complexes exhibited significant liver and heart uptake in rats and dogs. One complex in particular, 3-hydroxy-2-methyl-1-(p-nitrophenyl)-4-pyridinone labeled with Ga(III), showed accumulation in the brain of normal rabbits which increased over time. Z. Zhang et al., *Nuclear Medicine and Biology*, **1992**, *19*, 327-335.

^{68}Ga-[N,N,N',N'-tetrakis-(2-hydroxy-3,5-dimethylbenzyl)ethylenediamine], a neutral lipophilic hexachelated compound, exhibited significant myocardial uptake; however the brain uptake was insufficient to allow for measurement of cerebral blood flow. S.L. Madsen et al., *Nuclear Medicine & Biology, International Journal of Radiation Applications & Instrumentation - Part B,* **1992**, *19*, 431-44.

There has been some success in using chelates of other radionuclides, such as technetium-99m for delivery to the brain.

U.S. Patent No. 5,585,468 to Coughlin et al. describes bifunctional throurea-containing chelating agents labeled with Tc-99m. This disclosure suggests that small sized peptides can readily permeate the blood brain barrier and mentions the use of ^{68}Gallium for positron emission tomography (PET).

U.S. Patent No. 5,358,712 to Efange et al., describes analogs of vesamicol radiolabeled with ^{125}I, ^{123}I, or Tc-99m which penetrate the blood brain barrier for evaluating cholinergic innervation.

U.S. Patent Nos. 5,026,913 and 5,080,884 to McBride et al. describe a N_2S_2-type (2 nitrogens, 2 sulfurs) complex of a radioactive metal with a benzene ring having two 2-mercapto-2-methylpropylamine substituents for brain uptake. Brain uptake of Tc-99m complexes was shown. Other radioactive metals listed are isotopes of indium, ruthenium or gallium.

U.S. Patent No. 5,071,636 to Yamauchi et al. describes a N_2S_2-type polyaminediol compound chelated with Tc 99m which can be retained in the brain. The patent also lists gallium -67, gallium -62, thallium -201, indium -111, zinc -62 and copper -62 for use diagnostically in the chelates.

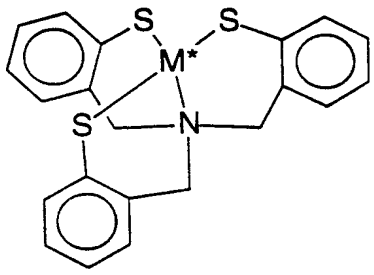
U.S. Patent Nos. 4,963,688 and 5,155,227 to Bodor describe a N_2S_2-type dihydropyridine and pyridinium salt type redox carrier for delivery of a radionuclide, particularly technetium-99m, to the brain. The patents also indicate that cobalt-57, gallium-67, gallium-68, indium-111, and indium-111m can be used diagnostically in the described chelates.

Other research has produced metal chelates without radionuclides. A tris (2-mercaptobenzyl)amine ligand is described by N. Govindaswamy, D.A. Quarless, Jr. and S.A. Koch, "New Amine Trithiolate Tripod Ligand and Its Iron(II) and Iron(III) Complexes", *Journal of the American Chemical Society*, **1995**, *117*, 8468-8569. The article describes complexation of the ligand with Fe(II). When Fe(III) was complexed, an additional 1-methylimidazole moiety was added to the ligand. The article does not suggest radiolabeling nor gallium complexes, nor any diagnostic or therapeutic use. Indeed, iron chelates are not generally used for diagnostics and therapeutics.

Accordingly, it is an object of the invention to provide radionuclide labeled compounds which can be of use in pharmaceuticals and for imaging of internal organs, particularly in brain and heart, with good uptake and retention.

SUMMARY OF THE INVENTION

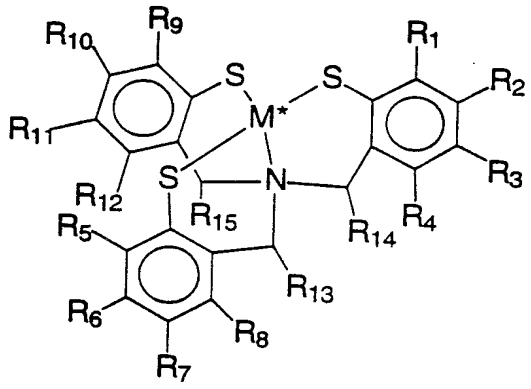
The invention provides radiolabeled complexes comprising a radionuclide and a ligand tris (2-mercaptobenzyl)amine, pharmaceutically acceptable salts of the complexes, or analogs of these complexes. A preferred complex can be illustrated by formula I.



Formula I

wherein M* is a radionuclide.

The ability of the compounds to form stable complexes with radionuclides results from the relative positions of the nitrogen and sulfur atoms. The compounds contain sufficient hydrogens for a stable molecule. Oxygen or selenium atoms can be substituted for one or more of the sulfur atoms in the tris(2-mercaptobenzyl)amine ligand. In addition, a hydrogen on the ligand tris(2-mercaptobenzyl) amine may be substituted by organic or inorganic groups without significantly affecting the stability of the complex. Substitutions are illustrated by formula II.



Formula II

Accordingly, one or more of the hydrogens may be independently substituted by an organic or inorganic substituent designated R_{1-15}. R_{1-15} can be independently selected from the group consisting of hydrocarbyl such as alkyl, aryl, alkylaryl or aryl- alkyl or heterohydrocarbyl which also includes oxygen, sulfur and/or nitrogen atoms in addition to carbon atoms; S-R_a; OR_b ; NR_aR_b; where R_a and R_b are independently H or alkyl of one to 10 carbons; or halo (F, Cl, Br, I). An alkyl may include one to 10 carbons or carbon-sized heteroatoms, such as oxygen, sulfur or nitrogen. Aryl substituents include five to ten carbons or heteroatoms such as oxygen, sulfur or nitrogen. In a preferred embodiment, R_{1-15} is hydrogen.

Therefore, organic substituents include, e.g., alkyl or heteroalkyl including esters, amides, ketones, carboxylic acid, etc.; these designations depending upon whether the substituent attaches to the ligand through a carbon of the substituent or a heteroatom of the substituent. Moreover, any of the carbons of the alkyl group may be separated from each other or from the ligand by groups such as carbonyl, oxycarbonyl, oxy, amino, thio, carboxy, etc. Alkyl groups may also terminate with groups such as halo, hydroxy, amino, thio, carboxy, etc. The term hydrocarbyl or heterohydrocarbyl as used herein means the monovalent moiety obtained upon removal of a hydrogen from a parent hydrocarbon.

Alkyls may be branched or unbranched and include, for example, methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, tert-butyl, isobutyl, octyl, nonyl or decyl. The alkyl groups may, in whole or in part, be in the form of rings such as cyclopentyl, cyclohexyl, cycloheptyl and cyclohexylmethyl.

Aryl substituents are aromatic groups, typically phenyl, but also may be other aryl group, for example, pyrrolyl, furanyl, thiophenyl, pyridyl, thiazoyl, etc. The aryl group may be further substituted by an inorganic, alkyl or other aryl group, these groups as described above.

The complexes are useful for diagnostic or therapeutic applications which involve localization of the complex in internal bodily organs. The radiolabel can then be detected by detection means, e.g., emission tomography. The complex is particularly useful for localization and retention in the brain and heart as well as other organs. The ligand can be termed S_3N, and the complex can be termed M*-S_3N. The preferred radioisotopes include ^{66}Ga, ^{67}Ga and ^{68}Ga..

Delivery of compounds, including radiopharmaceuticals to the brain is difficult because of limitations by transport and metabolism factors, particularly the blood brain barrier. The endothelial brain capillary wall is a functional blood brain barrier which prevents delivery of many pharmaceutical agents to the brain. A gallium complex according to the invention meets all the requirements of a brain imaging agent: it has a low molecular weight, it is neutral, lipophilic, and stable to transchelation to transferrin. Along with high extraction in the brain, the complex also shows significant heart uptake, and also good uptake in other organs.

DETAILED DESCRIPTION OF THE INVENTION

Radiopharmaceuticals contain isotopes which can be detected in the body. Once uptake in a bodily organ has occurred, retention of the isotope by the organ permits detection by known detection means such as emission tomography.

Emission tomography is used to measure physiological functions of organs and related biochemical processes. The two modes of emission tomography are single-photon emission computed tomography (SPECT) and positron emission tomography (PET). SPECT uses radionuclides that emit a single photon of a given energy and these include gamma ray emitters such as ^{67}Ga, ^{97}Ru, ^{99m}Tc, ^{111}In, ^{123}I, ^{131}I, ^{203}Pb, and others. PET uses radionuclides known as positron emitters such as ^{11}C, ^{15}O, ^{18}F, ^{55}Co, ^{64}Cu, ^{68}Ga, ^{75}Br, ^{89}Zr, ^{124}I, and others, for coincidence detection in which the positron and electron annihilate each other to form two photons at 180° angle from each other. In SPECT and PET, transverse section reconstruction of the radionuclide distribution

within the body is obtained by acquiring images of multiple slices of the organ or the whole body.

Radionuclides suitable for use in the invention include any radioactive metal which has physical and chemical characteristics useful for nuclear medicine applications. Preferred are ^{99m}Tc, ^{186}Re, ^{188}Re, ^{111}In, ^{113m}In, ^{64}Cu, ^{66}Ga, ^{67}Ga and ^{68}Ga. More preferred is Ga, particularly for applications in the brain and heart.

Radioactive congeners of gallium (^{67}Ga, ^{68}Ga) produce ionizing radiation suitable for diagnostic nuclear medicine. Gallium-68 is a positron emitting isotope with a half-life of 68 min and a high abundance of positrons (89%), making it ideal for PET imaging. The half-life allows chemical manipulation but limits the dose received by the patient. Unlike many of the other isotopes used for PET which are cyclotron produced, Gallium -68 can be obtained from a ^{68}Germanium/^{68} Gallium generator. Germanium-68 ($t_{1/2}$=280 days) is loaded onto a tin dioxide column which decays by electron capture to Ga-68. C. Loch et al., *Journal of Nuclear Medicine,* **1980**, *21*, 171-173. The generator can be eluted several times a day with 1 N HCl, producing Gallium-68 in a form ready to be used to synthesize radiopharmaceuticals. The long half-life of the parent allows for a shelf-life of 1.5-2 years for the generator. This generator system enables hospitals which are unable to afford the expense of a cyclotron to perform PET imaging. The use of ^{68}Ga generated in this way is much more convenient than using, e.g., ^{67}Ga which must be cyclotron-produced. However, either of these can be used in the invention. ^{66}Ga ($t_{1/2}$=9.5), another positron emitter, with its high energy photons could be used for imaging and radiotherapy.

The Lewis acidity of the radioactive metal ions makes them subject to hydrolysis at physiological pH. The metal radionuclides must therefore be ligated by ligands that offer highly stable complexation. The complexes of the invention are stable *in vivo* and *in vitro.* By stable is meant having reasonable lifetimes for use in nuclear medicine. The complexes are small, neutral and lipophilic and can easily be formed in high yield with a purity over 95%.

The ligands can be prepared by methods described by N. Govindaswamy, D.A. Quarless, Jr., and S.A. Koch, *J. Am. Chem. Soc.* **1995**, *117*, 8486-87 and by N. Govindaswamy, Ph.D. dissertation, State University of New York at Stony Brook, NY, December 1995.

In one synthesis method, for example, a thiol-protected benzylamine is reacted with 2 equivalents of the corresponding benzyl bromide in CH_3CN with K_2CO_3 to give the tertiary amine. Removal of the thiol protecting groups can be accomplished by reaction with sodium in liquid ammonia to give the ligand tris (2-mercaptobenzyl)amine (S_3N).

To prepare the radionuclide complex, in one embodiment, ligand solution is prepared by dissolving the S_3N ligand in water-free alcohol. The alcohols are preferably lower organic alcohols such as ethanol or methanol. Ethanol is preferred. $^{68}GaCl_3$ is eluted from a $^{68}Ga/^{68}Ge$ generator with acid, dried, and mixed with the ligand solution. After reaction at ambient temperature, e.g., room temperature for a sufficient time, e.g., 10-15 minutes, the complex is recovered. In another synthesis, the Ga-S_3N can be made by providing a saturated acetonitrile solution of tetraphenylphosphonium tetrachlorogallate $[Ph_4P][GaCl_4]$ prepared by dissolving Ga_2O_3 in hot concentrated HCl and adding ethanolic solution of tetraphenylphosphonium bromide (Ph_4PBr) to yield tetraphenylphosphonium tetrachlorogallate $[Ph_4P][GaCl_4]$, which is then added to trilithiated tris benzylthiolato amine in alcohol to yield GaS_3N.

In vivo diagnostic uses include as non-limiting examples, imaging of brain, heart, lung, liver and tumors. The complex of the invention was shown to cross the blood brain barrier sufficiently for measurement of cerebral blood flow. As non-limiting examples, the brain imaging is useful for screening for the presence of tumors, both benign and malignant, for detection of cerebral metastasis, evaluation of cerebrovascular disease, detection of intracranial injury due to trauma such as subdural hematoma or intracerebral hemorrhage, localization of intracranial abcesses,

localization of arteriovenous malformations, evaluation of intracranial diseases such as meningitis and encephalitis, determination of legally defined brain death, detection of sites of epilepsy or seizures, determination of tissue viability after stroke, etc. Good uptake and retention also occur in the heart allowing myocardial imaging, for example, to determine areas of infarction and tissue viability.

In addition, autoradiography shows that the complex behaves similarly to Cu-PTSM which goes to tumors indicating that the complex of the invention may have tumor uptake.

Using known methods of attachment, the complex can also be used to label peptides, antibodies and estrogens, e.g., with Ga-68, Ga-67 or Tc-99m for tumor diagnosis and Ga-66, Cu-64, Re-186 or Re-188 for therapy.

Test results showed that ^{68}Ga-S_3N crossed the blood brain barrier, e.g., with a brain:blood ratio of 3.8± 0.3 at one hour post injection in rats. The heart:blood ratio at one hour, e.g., was 11.0±0.5. The heart uptake, e.g., was 1.04% dose/organ, with 1.3% dose/g at 30 min and 0.75% dose/g at one hour. The heart uptake values appeared to be consistently similar in rat, hamster and canine. The complex showed high liver uptake, e.g., initially of 72% dose/organ that quickly washed out to 21% dose/organ at one hour.

In vitro serum stability determined in rat serum incubated at 37°C. showed the ^{68}Ga-S_3N complex to be greater than 95% intact at 2 hours. *In vivo* ^{68}Ga-S_3N remains stable to quickly clear from the blood and to prevent transchelation of gallium to serum proteins. By 30 min low amounts of activity remained in the blood and its extracts contained three radioactive complexes; one the original intact complex and two less lipophilic metabolites.

The ^{68}Ga-S_3N complex cleared rapidly from the blood, and demonstrated very high liver uptake at early times post-injection which cleared over time. This rapid

clearance from the blood and subsequent clearing from the liver indicates this complex is stable *in vivo*. It is known that unstable gallium complexes tend to clear slowly from the blood and to accumulate radioactivity in the liver.

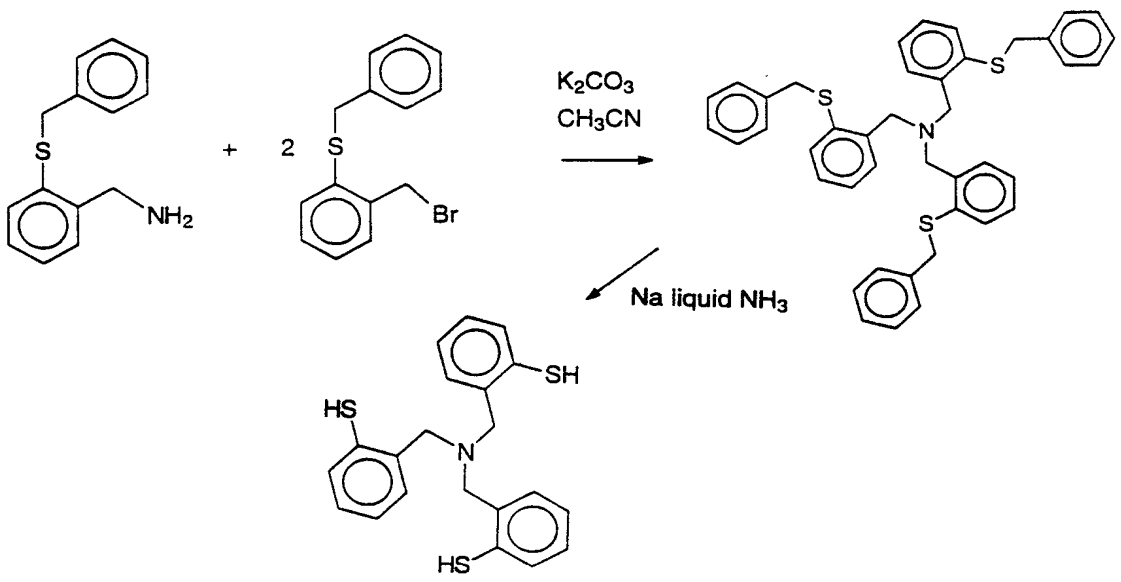
The pattern of biodistribution for the ^{68}Ga-S_3N complex is especially interesting. ^{68}Ga-S_3N behaves atypically of other perfusion agents in that the brain uptake is not first pass but accumulates over time as demonstrated in Table 1. Normally, a perfusion agent exhibits its highest uptake in the organ of interest a few minutes post injection when the agent's concentration is highest in the blood. A high lung intake and non-clearance from this organ is also interesting. While it is not intended to be bound by theory, there are various possible explanations for the gradual increase in brain uptake of ^{68}Ga-S_3N over time. ^{68}Ga-S_3N may be re-extracted into the brain and lung after originally being taken up by other tissues such as the liver and then released or ^{68}Ga-S_3N may be metabolized by the liver forming a metabolite that is able to cross the blood brain barrier. Preliminary metabolism studies in rats show that ^{68}Ga-S_3N is metabolized in the liver forming three less lipophilic metabolites. The brain distribution of ^{68}Ga-S_3N , as shown by autoradiography, is that expected of a lipophilic agent.

The invention may be illustrated by the following non-limiting examples:

EXAMPLE 1

Preparation of Ligand

A tetradentate amine trithiolate ligand, tris (2-mercaptobenzyl)amine (S_3N) was prepared by reacting thiol-protected benzylamine with 2 equivalents of the corresponding benzyl bromide in CH_3CN with K_2CO_3 to give the tertiary amine. Removal of the thiol-protecting groups by reaction with Na in liquid ammonia gave the ligand S_3N which was isolated as the hydrochloride salt.



Another synthesis is as follows:

tris-(2-bromobenzyl)amine from ammonium acetate

10.00g (60 mmol) of 2-bromobenzylbromide was placed in a 500 mL flask containing 150mL of acetonitrile and 10.0g of K_2CO_3. To this was added 1.44g (20mmol) of ammonium acetate. The solution was stirred at room temperature for 6 hrs. and then refluxed overnight. The reaction mixture was cooled and the solvent was evaporated. 150mL of water was added to the residue and this aqueous phase was extracted with ether (150mL x 2). The organic phases were combined and dried over Na_2SO_4. Evaporation of the solvent gave tris-(2-bromobenzyl)amine in 72% yield.

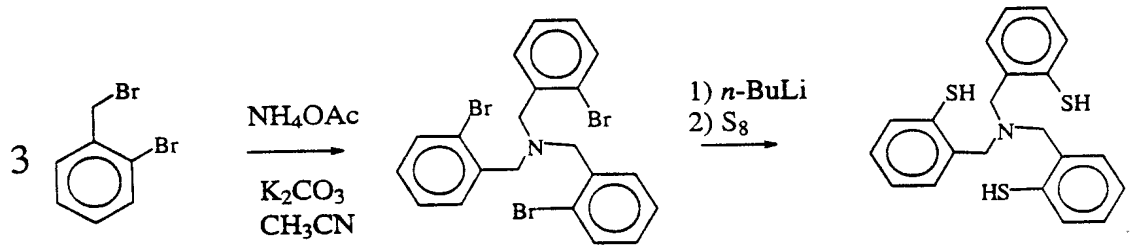
^{1}H NMR (δ, $CDCl_3$); 3.79ppm (s, 6H CH_2), 7.07ppm (t,3H, Ar), 7.25ppm (t, 3H, Ar), 7.49ppm (d, 3H, Ar), 7.67ppm (d, 3H, Ar)

tris(2-mercaptobenzyl)amine hydrochloride

Tris-(2-bromobenzyl)amine, (NBr_3), (15.0g, 28.6 mmol) was partially dissolved in 100 mL of diethyl ether cooled in a dry ice/acetone bath (-78°C). A volume of 38.0

mL of 2.5 M n-BuLi (95.0 mmol) was added dropwise to the reaction mixture. The mixture was stirred at -78°C for several minutes preceding the removal of the bath. Over the course of about 30min, the disappearance of the undissolved starting material was monitored. As soon as starting material has completely dissolved, the reaction mixture was returned to the dry ice/acetone bath. Sublimed sulfur, S_8 (3.05g, 11.9 mmol) was slowly added to the homogeneous organolithium reagent at -78° C. After several minutes the dry ice bath was removed. A white precipitate formed as the clear reaction solution equilibrated to ambient temperatures. The while precipitate was extracted in 100 mL of a 3.80% NaOH solution. The aqueous phase was washed with three 40mL portions of methylene chloride and then acidified to pH 1 with aqueous hydrochloric acid. The copious white solid was filtered, washed with two 10 mL portions of cold methanol and 15 mL portion of cold methylene chloride and dried under vacuum (9.6g, 80% Yield).

^{1}H NMR ($CDCl_3$): 4.64 ppm -CH_2, 7.32 ppm, 6H, (Ar), 7.42 ppm, dd (3H, (Ar)), 8.04 ppm d(3H (Ar)).



EXAMPLE 2

Preparation of Gallium Complexes

Tris(mercaptobenzyl) amine hydrochloride (0.55 g 1.3 mmol) as prepared in Example 1 and lithium wire (0.036g, 5.2 mmol) were reacted in 20 mL of methanol. A saturated acetonitrile solution containing 0.72g (1.3mmol) of $[Ph_4P][GaCl_4]$ was added. The solution mixture immediately produced a fine precipitate. The reaction

mixture was stirred for an additional one half-hour and cooled at -20°C for 12 hours. A 65% yield (0.38g, 0.85 mmol) of white powder, was isolated by filtration and dried in vacuum. The compounds were recrystallized from dimethylformamide/water.

^{1}H NMR ($CDCl_3$): 3.42 ppm (d, 3H,-CH_2), 4.63 ppm (d,3H,-CH_2), 7.16-7.30 ppm (9H, Ar), 7.52 ppm (d, 3H, Ar).

Characterization of Ga-S_3N by X-ray Crystal Structure Determination

Ga-S_3N was crystallized from DMF/H_2O solution. Its structure was determined by single crystal X-ray crystallography. The unit cell for Ga-S_3N was determined for formula unit $GaS_3ON_2C_{24}H_{25}$ of formula weight 523.37, a =10.448(1) Å, b = 14.3817(8) Å, c = 15.677(2) Å, alpha = 90°, beta = 92.310(6)°, gamma = 90°, Volume = 2353.7(4) Å^3; Z=4, monoclinic space group $P2_1/c$. The structure consisted of discrete monomeric Ga-S_3N molecules in which the Ga^{3+} ion was coordinated to three sulfur atoms and the amine nitrogen atom of the ligand. Ga-S_3N had a distorted tetrahedral coordination with average S-Ga-S angles of 115.5(7)° and the average N-Ga-S angles of 102.4(2)°. The average Ga-S bond distance was 2.234(5) Å and Ga-N bond distance was 2.053(6) Å.

EXAMPLE 3

Preparation of Radiolabeled Complex

The ligand solution was prepared by dissolving ~ 1 mg of ligand in 1 mL of ethanol that had been degassed for 15 min with argon. ^{68}Ga-Cl_3 (15-20 mCi) was eluted from a ^{68}Ge/^{68}Ga generator with 3 mL of 1N HC1. The ^{68}Ga-Cl_3 was evaporated to dryness with a heat gun under a stream of nitrogen, redissolved in 1 mL of ethanol and degassed for 10 min with argon. 100 μg (80-120 μL) of the ligand solution was then added to the dried $^{68}GaCl_3$ and incubated at room temperature for 10 min. Quality control was determined by radio-TLC (thin layer chromatography) on C-18 plates developed in 90% methanol: 10% water, and by radio-TLC on silica plates

developed in 100% methanol. The product was purified by addition of 3 mL of saline and subsequent loading onto a preconditioned C18 Sep-Pak® Light (Waters Corp., Milford, MA) (prepared by washing with 3-5 mL of ethanol, followed by 3-5 mL of normal saline). The Sep-Pak was then washed with 3 mL of saline. The complex was eluted with 400 μL of ethanol. Saline was then added to the eluent to give a resultant solution of 85% saline/15% ethanol and the % complex determined by TLC. Complex charge was determined by electrophoresis. Comparison compound ^{64}Cu-PTSM (Cu-pyruvaldehyde bis(N^4-methylthiosemicarbazone) was prepared and analyzed as described by K. Matsumoto et al., *Nuclear Medicine and Biology,* **1992**, *19,* 39-44, with the exception that PTSM was dissolved in DMSO rather than ethanol. The comparison compound is used in Example 6 below.

The radiochemical yield of ^{68}Ga-S_3N was consistently >95%. ^{68}Ga-S_3N migrated with an R_f=0.8 on silica plates developed in 100% methanol and an R_f=0.4 on C18 plates developed in 90% methanol: 10% water (v/v). In both systems ^{68}Ga-Cl_3 remained at the origin.

In electrophoresis the ^{68}Ga-S_3N complex migrated with an R_f=0.01. ^{111}In-DTPA was used as a standard and migrated with an R_f=0.24. These results indicate that the ^{68}Ga labeled S_3N complex was neutral.

Determination of Partition Coefficients

The partition coefficients (log P; n=3) were determined by adding 5-20μL of labeled complex to a solution containing 2 mL of octanol and 2 mL of water (obtained from saturated octanol water solutions). The resulting solutions were then vortexed and centrifuged for 5 min at 2400 rpm. 1 mL of octanol was removed and back extracted with 1 mL of water, vortexed and centrifuged as before. 500 μL of octanol and 500 μL of water were removed and counted. 500 μL of water was added to the 500 μL of octanol, vortexed and centrifuged as before. 200 μL of octanol and 200 μL of water were removed and counted. The partition coefficient was calculated as a

ratio of counts in the octanol fraction to counts in the water fraction per extraction. The average log P value of the two back extractions is reported.

The octanol-water partition coefficient or log P of ^{68}Ga-S_3N was determined to be 1.8±0.1. Dischino and coworkers studied the relationship between the lipophilicity of a compound and its extraction by the brain, Dischino et al., *Journal of Nuclear Medicine*, **1983**, *24*, 1031-1038. Their results showed that a radiopharmaceutical designed to measure blood flow should have a log P value of between 0.9 and 2.5. Based on these results the log P value of 1.8±0.1 obtained for ^{68}Ga-S_3N is right in the middle of the range suggested by Dischino and therefore should freely diffuse across the blood brain barrier.

EXAMPLE 4

Serum Stability Studies

In vitro serum stability studies were conducted by placing 100 μCi of ^{68}Ga-S_3N in 500-1000 μL of freshly drawn rat serum and incubating at 37°C. Samples were removed at various time points and analyzed by both C18 and silica radio-TLC.

The *in vitro* stability results of ^{68}Ga-S_3N in rat serum showed that ^{68}Ga-S_3N remained over 95% intact at least to 2 hours.

EXAMPLE 5

Biodistribution Studies

All animals studies were performed in compliance with guidelines set forth by the Washington University Animal Studies Committee. Mature female Sprague-Dawley rats (n=4 per time point) weighing 150-200g were anesthetized with Metofane (2, 2-dichloro-1, 1 difluoro-1-methoxyethanol) and injected with 15-20 μCi of ^{68}Ga-S_3N in a volume of 150-200 μL 85% saline/15% ethanol via the tail vein. The rats were sacrificed and the lung, liver, spleen, kidney, heart and brain were removed from

each animal, placed on absorbent paper and weighed. Blood samples were collected directly and weighed. Blanks and standards were prepared and counted along with the samples in order to calculate the percent injected dose per gram of tissue (%ID/g), percent injected dose per organ (%ID/organ) and to correct for physical decay. The results are shown in Table 1 below.

TABLE 1

ORGAN	2 min	15 min	30 min	50 min
Blood	8.5 ± 1.9	0.98 ± 0.20	1.2 ± 0.1	0.76 ± 0.2
Brain	0.13 ± 0.03	0.25 ± 0.04	0.42 ± 0.05	0.40 ± 0.07
Heart	1.0 ± 0.3	0.77 ± 0.19	0.95 ± 0.14	0.49 ± 0.13
Lung	3.0 ± 1.0	2.7 ± 0.7	4.4 ± 0.7	6.2 ± 1.4
Liver	72 ± 7	55 ± 15	44 ± 5	21 ± 1
Spleen	5.5 ± 1.8	2.5 ± 1.1	0.94 ± 0.14	0.25 ± 0.04
Kidney	0.34 ± 0.03	0.56 ± 0.10	0.72 ± 0.16	0.47 ± 0.10

The biodistribution results in rats for ^{68}Ga-S_3N showed that ^{68}Ga-S_3N cleared rapidly from the blood with an initial high liver uptake (72±7% ID/organ at 2 min) followed by rapid washout (21±1% at 60 min). High uptake was also seen in the lungs (3±1% at 2 min) which accumulated over time (6±1% at 60 min) and the spleen (5±2% at 2 min) which cleared with time (0.25±0.04% at 60 min). Significant uptake was observed in the brain (0.13±0.2% at 2 min) which increased over time to (0.40±0.07% at 60 min) with brain:blood ratios increasing from 0.11 at 2 min post injection to 3.8 at 60 min post injection. Uptake was also seen in the heart (1.04±0.26% at 2 min) decreasing to (0.50±0.12% at 60 min) with a heart:blood ratio of 2.5 at 2 min post injection and 11 at 60 min post injection.

A hamster biodistribution was carried out in the same manner as the rat biodistribution, the only difference being 14 μCi of ^{68}Ga-S_3N in 100μL of 85% saline; 15% ethanol were injected intracardially.

The biodistribution results in hamsters for ^{68}Ga-S_3N showed that the complex cleared rapidly from the blood in hamsters as observed in rats, however, the blood values for hamsters (3.5 ± 0.4% at 30 min) at all but the initial timepoint were higher than those observed in rats 1.2 ± 0.1% at 30 min). The high initial liver uptake that was seen for rats was also observed for hamsters (94 ± 11% at 5 min). The washout from the liver was significantly slower in hamsters (70 ± 5% at 60 min). The lung uptake in hamsters was similar to that observed in rats at the early time point (3 ± 1% at 5 min), however, it did not increase as observed in rats but actually decreased (2.6 ± 0.2% at 60 min). Spleen uptake was high initially (2.2 ± 0.6% at 5 min) but decreased over time (0.11 ± 0.03% at 60 min). Significant uptake was observed in the heart (1.04 ± 0.8% at 5 min) decreasing over time (0.30 ± 0.08% at 60 min) comparable to that observed for rats. Significant uptake was observed in the brain (0.13 ± 0.03% at 5 min) that increased over time (0.25 ± 0.04% at 60 min), but with uptake lower than that observed in rats.

EXAMPLE 6

Autoradiography Experiments

Mature female Sprague-Dawley rats were injected with approximately 400 μCi of ^{68}Ga-S_3N and 47 μCi of comparison compound ^{64}Cu-PTSM (prepared in Example 3) as regional perfusion marker via the tail vein, anesthetized and sacrificed at 30 min post injection. The brain was removed, frozen and cut into 1 mm thick slices and Ga-68 images were obtained. After decay of Ga-68, Cu-64 images were obtained.

In a separate experiment for comparison purposes, a previously published procedure for the preparation of alkyl iodides was adapted to synthesize C-11-ethanol. Dischino et al., *J. Nucl. Med.* **1983**, 24, 1031-1034. Briefly, C-11 CO_2 was bubbled through 1.0 ml dry ether containing 200μL of 3 M methylmagnesium bromide, followed by addition of 200 μL of 1M LAH and 200μL of ether. The mixture was then heated at 100°C for 3 min. The solvent was evaporated and 1.0 mL of 1.0 N HCl was added to decompose the lithium complex. ^{11}C-ethanol was distilled at 105°C

and collected in 1.5 mL of saline. 180-200 μCi (200 μL) were injected via the tail vein into an anesthetized rat, and the rat was then sacrificed at 1 min post injection. The brain was removed and prepared as above and imaging was performed.

The brain distribution in normal rats of ^{68}Ga-S_3N was compared to that of the two known blood flow tracers, ^{64}Cu-PTSM and ^{11}C-ethanol. Images obtained with ^{68}Ga-S_3N, ^{64}Cu-PTSM and ^{11}C-ethanol autoradiography scans were very similar. The only notable difference was the higher uptake of ^{68}Ga-S_3N in the cortex in the back of the brain. The scan of brain slices containing ^{11}C-ethanol were notably different from those of ^{68}Ga-S_3N. These results reflect the similar lipophilicities of ^{68}Ga-S_3N and ^{64}Cu-PTSM.

EXAMPLE 7

Primate Imaging Study

A male nemestrina monkey weight 16 kg was anesthesized with 300 mg of ketamine, 10 mg of xylazine and 0.2 mg of atropine sulfate injected intramuscularly. The study was approved by the animal studies committee. The animal was injected with 25 mCi of 0-15 labeled water to measure brain blood flow and 20 mCi of carbon monoxide was administered by inhalation to determine blood volume. 3.4 mCi of ^{68}Ga-S_3N was administered intravenously. One minute images were acquired for 15 minutes and then nineteen 5 minute images acquired for a total acquisition time of 110 minutes.

Imaging studies were performed on a PET scanner. The reconstructed resolution was approximately 6 mm in the transverse plane and about 4.5 mm in the axial direction; axial sampling was about 3.4 mm. The animal was fasted overnight prior to the study but allowed free access to water up to two hours before the study. Magnetic resonance imaging studies were performed on the monkey with the animal fully anesthetized. Careful monitoring of the animal's condition including temperature,

end-tidal CO_2, respiratory rate, electrocardiogram and pulse provided online information during the MRI scans. There were no signs of distress throughout the PET or MRI procedure.

PET brain images obtained with ^{68}Ga-S_3N and ^{15}O-water were similar to the autoradiography results in the rat brain, with the brain uptake of ^{68}Ga-S_3N showing greater uptake in the cortex at the back of the brain. Higher uptake was also demonstrated in the clivus and the temporal mandibular joints.

EXAMPLE 8

Canine Imaging Study

An adult male canine weighing 27 kg was premedicated with 0.75 mg atropine and 5 mg of acepromazine subcutaneously. After 30 min, anesthesia was induced with 12.5 ml 5% sodium thiopental given IV via an established right cephalic vein catheter. The animal was intubated with a 9 french endotracheal tube and mechanically ventilated (tidal volume =15 ml/kg) using 1.5-2.5% inhalant isoflurane. Anesthesia was maintained throughout the procedure. The animal was injected with 21 mCi of O-15 labeled water and 22 mCi of carbon monoxide. 5.97 mCi of ^{68}Ga-S_3N was injected through the established right cephalic vein IV line. Acquisitions were acquired for 5 min for a total of 90 minutes. Imaging stuides were performed on a PET scanner as in Example 5.

In heart imaging studies obtained with ^{68}Ga-S_3N and ^{15}O-water, the high lung uptake observed in both rats and hamsters was also observed in canines. The lung uptake greatly decreased over time differing from that observed in either rats or hamsters. The liver intake remained fairly constant over time. The liver uptake in both rats and hamsters declined over time. The clearance from the myocardium was comparable to that observed in both rats and hamsters.

EXAMPLE 9

Preparation of Indium Complexes

A methanol solution containing 1.2 mmol of the tris lithium thiolate of the S_3N ligand was combined with $InCl_3$ (0.25g, 1.1 mmol) and the reaction mixture stirred for one half hour. The solvent was removed by vacuum. The residue was redissolved in 5mL of DMF, which was layered with distilled water and cooled at -20°C. Crystalline needles of the product In(S_3N)(dimethylformamide) separated from the solution overnight in 76% yield.

^{1}H NMR (CD Cl_3): 2.91 ppm (6H, -CH_3), 2.98 ppm (6H, -CH_3, DMF), 3.18 ppm (3H, -CH_2), 7.12-7.15 ppm (6H, Ar (S_3N), 7.58 ppm (d, 3H, Ar (S_3N)), 8.05 ppm (s, 3H, Ar (S_3N)).

Serum Stability Studies

Serum Stability studies of In-S_3N were carried out as in Example 4. Stability constants were determined potentiometrically in 70% ethanol/water (v/v). The stability constant of In- S_3N was similar to that of Ga-S_3N. *In vitro* serum stability in rat serum incubated at 37° C showed the In S_3N to be 81% intact at 2 hours.

EXAMPLE 10

Determination of Coordination Number

The coordination scan technique was used to determine the coordination number of the Ga(III) S_3N complex and the In(III)S_3N complex. The coordination scan technique is described by Hancock et al. in Hancock, R.D. *Progress in Inorganic Chemistry* **1989**, 37, 187-291; Thöm, V; Fox, C.C.; Boeyens, J.C.A.; Hancock, R.D. *Journal of the American Chemical Society* **1984**, 106, 5947-5955; Hancock, R.D. *Pure and Applied Chemical* **1986**, 58, 1445-1452; and Hancock, R.D. *Accounts of Chemical Research* **1940**, 23, 253-257. Hancock and coworkers have successfully

utilized molecular mechanics to determine the relationship between ligand selectivity and metal ion size. The technique utilized in these studies is the calculation of the complex strain energy as a function of the M-L bond length. A related technique is the "coordination scan," in which a series of energy curves are generated by minimizing strain energy of complexes with various numbers of coordinated water molecules and M-L bond lengths. The preferred coordination number of the metal is determined by the position of the ionic radius in relation to the location of the intersection points. This technique has previously been found to successfully predict the coordianation number of various Ga(III) and In(III) complexes. Sun, Y.; Anderson, C.J.; Pajeau, T.S.; Reichert, D.E.; Hancock, R.D.; Motekaitis, R.J.; Martell, A.E.; Welch, M.J. *Journal of Medicinal Chemistry* **1966**, 39, 458-70. The starting structure was that of the previously reported Fe(III) x-ray coordinates. Govindaswamy, N.; Quarless, D.A., Jr.; Koch, S.A. *Journal of the American Chemical Society* **1995**, 117, 8468-8469. The metal in each complex was then adjusted to the different coordination numbers by covalently binding the appropriate number of waters to the metal. An important point to note is that SYBYL calculates water as having a strain energy of 0.00 kcal/mol, thus the waters added to the complex add no energy other than steric interactions with the ligand.

Force field parameters for bonds between N, O, and S donor atoms with Ga(III) were those developed for the modeling package SYBYL (Tripas SYBYL; 6.2 ed.; Tripos, Ed., Tripos Inc., St. Louis, 1995). The metal's ionic radius was effectively varied by systematically altering the M - N, M - S, and M - O equilibrium bond lengths in the following manner. The M - N bond length was assigned through the following relationship: eq. bond length = (M ionic radius) + 1.58 Å. Similarly, the M - O equilibrium bond lengths were assigned using: eq. bond length = (M ionic radius) + 1.41 Å. The M - S equilibrium bond lengths were assigned using: eq. bond length = (M ionic radius) + 1.76 Å. The initial metal ionic radius was set at 0.3 Å. The force constant for the bond stretching was kept at a constant value of 100 kcal mol^{-1} $Å^{-1}$. The complex was then minimized using this ionic radius, and the energy of the complex found. The ionic radius was then increased by 0.1 Å, the equilibrium bond

length modified to the new value, and the complex again minimized. This process was continued until the ionic radius had reached 1.5 Å; this range of 0.3-1.5 Å was sufficiently large that it covered the radii of all the possible coordination states. This procedure was then repeated for the complex with one water, two waters, and so on until all the possible coordination states were examined.

Plots of the complex energy versus metal ionic radius were then generated for each coordination number. The preferred coordination number of a metal is found by locating the preferred ionic radii on the x-axis of the plot and the lowest energy curve, which corresponds to the preferred coordination number. For Ga(III) the radius for a 4-coordinate environment is 0.47 Å, for 5-coordinate, 0.55 Å, and for 6-coordinate 0.62 Å.

The molecular mechanics technique referred to as the "coordination scan", which successfully predicts the coordination number of metal complexes, has been used to understand the behavior of Ga(III) and In(III) complexes with a series of multidentate thiolate ligands, EDASS, 4SS, 5SS, and 6SS. Y. Sun et al., *Journal of Medicinal Chemistry,* **1990**, 39, 458-70. The coordination scan indicated that this ligand would bind Ga(III).

The Ga(III) complex was predicted to be 4-coordinate in a tetrahedral geometry, thus fulfilling the coordination requirements of the metal. This prediction was later confirmed by x-ray crystallography. In(III) prefers to be 5 coordinate. The In(III) complex was shown to form predominantly a five coordinate complex in equilibrium with a four coordinate complex.

In conclusion, S_3N was successfully radiolabeled with Ga and In. The biodistribution of the GaS_3N complex showed it was rapidly taken up by the liver and exhibited high uptake in the heart and brain. Molecular mechanics calculations predicted that GaS_3N was kinetically stable. The high brain uptake of the complex was

also observed in a non-human primate, demonstrating the particular usefulness of this agent as a diagnostic PET tracer for cerebral blood flow.

While there have been described what are presently believed to be the preferred embodiments of the invention, those skilled in the art will realize that changes and modifications may be made thereto without departing from the spirit of the invention, and it is intended to claim all such changes and modifications as fall within the true scope of the invention.

CLAIMS:

1. A radiolabeled complex comprising tris(2-mercaptobenzyl)amine and a radionuclide.

2. The complex of Claim 1 wherein at least one hydrogen of the tris(2-mercaptobenzyl)amine ligand is independently substituted by

alkyl of one to about 10 carbon atoms or heteroatoms;

aryl of about 5 to about 10 carbon atoms or heteroatoms;

halo; S-R_a; OR_b; or NR_aR_b;

wherein heteroatoms are oxygen, sulfur, or nitrogen and R_a and R_b are independently hydrogen or alkyl of one to about 10 carbon atoms.

3. The complex of Claim 1 wherein oxygen or selenium replaces at least one of the sulfurs of the tris(2-mercaptobenzyl)amine.

4. A radiolabeled imaging agent comprising the complex of Claim 1.

5. A radiolabeled imaging agent comprising the complex of Claim 2.

6. A radiolabeled imaging agent comprising the complex of Claim 3.

7. The complex of Claim 1, wherein the radionuclide is gallium.

8. The complex of Claim 7, wherein the complex is capable of crossing the blood brain barrier.

9. The complex of Claim 8, wherein the complex is retained in the brain for a period of time sufficient for detection by detection means.

10. The complex of Claim 9 wherein the detection means comprises emission tomography.

11. The use of a radiolabeled complex comprising a radionuclide and tris (2-mercaptobenzyl)amine for diagnostic or therapeutic applications which involve localization and/or detection of the complex in internal organs.

12. The use according to Claim 11 wherein at least one hydrogen on the tris(2-mercaptobenzyl)amine is independently substituted by

alkyl of one to about 10 carbon atoms or heteroatoms;

aryl of about 5 to about 10 carbon atoms or heteroatoms;

halo; S-R_a; OR_b; or NR_aR_b;

wherein heteroatoms are oxygen, sulfur, or nitrogen and R_a and R_b are independently hydrogen or alkyl of one to about 10 carbon atoms.

13. The use according to Claim 11 wherein oxygen or selenium is independently substituted for at least one sulfur of the tris(2-mercaptobenzyl)amine.

14. The use according to Claim 11 wherein the radionuclide is gallium.

15. The use according to Claim 14, wherein the internal organ is brain or heart.

16. The use according to Claim 11, wherein the diagnostic application is imaging.

17. The use according to Claim 12, wherein the diagnostic application is imaging.

18. The use according to Claim 13 wherein the diagnostic application is imaging.

19. The use according to Claim 11, wherein the imaging comprises introducing a diagnostically effective amount of the complex into the body and thereafter detecting the radiolabel with detection means.

20. The use according to Claim 11 wherein the detection means is emission tomography.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/09631

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 51/04; C07F 5/00
US CL :424/1.65, 1.11, 1.85; 534/10, 14

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/1.65, 1.11, 1.85; 534/10, 14

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CAPLUS
search terms: mercaptobenzylamine, S3N, radionuclide, radiodiagnostic, radiolabel

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y,P	US 5,861,140 A (PENG ET AL.) 19 January 1999, see column 4 and column 8.	1-20
X	Database CAPLUS, Accession Number 141326, WELCH, M.J. et al. New Metal Complexes of Indium, Gallium and Copper for Medical Applications, Book of Abstracts, 215th ACS National Meeting, March 1998.	1-20
Y	MOTEKAITIS, R.J. et al. The Ga(III) and In(II) complexes of tris(2-mercaptobenzyl)amine, Abstract Number 783, Book of Abstracts, 213th ACS National Meeting, April 1997.	1-20

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

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Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer Michael G. Hartley Telephone No. (703) 308-1235

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